# PATENT ABSTRACTS OF JAPAN

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## (54) METHOD FOR ISOLATION AND PURIFICATION OF CHONDROITIN SULFATE

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a technology which can supply chondroitin sulfate in large quantity and at low cost from a raw material of nosal cartilages of fishes, especially of salmon heads.
SOLUTION: A technology to obtain the powder of chondroitin sulfate comprises the steps of: alkali-treating fish's nosal cartilages containing chondroitin sulfate, and optionally, treating it by means of proteolytic enzyme, thereby to give an aqueous solution containing chondroitin sulfate, diluting this aqueous solution with water, thereafter repeating the concentrating operation of chondroitin sulfate by ultrafiltration treatment, and either drying the concentrated liquid as it is obtained or depositing chondroitin sulfate by adding ethanol to the concentrated liquid obtained.

LEGAL STATUS

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CLAIMS

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[Claim(s)]
[Claim 1] The separation purification approach of the chondroitin sulfate characterized by obtaining the water solution which carries out alkali treatment of the tissue of the animal containing chondroitin sulfate, and contains chondroitin sulfate, carrying out ultrafiltration processing of this water solution, and performing concentration and purification of chondroitin sulfate.
[Claim 2] The separation purification approach of the chondroitin sulfate according to claim 1 which obtains the water solution which processes with proteolytic enzyme and contains chondroitin sulfate after alkali treatment, and carries out ultrafiltration processing of the water solution.
[Claim 3] The separation purification approach of chondroitin sulfate according to claim 1 or 2 of obtaining chondroitin sulfate powder by drying the concentration liquid of the chondroitin sulfate obtained by ultrafiltration processing.
[Claim 4] The separation purification approach of the chondroitin sulfate according to claim 1 or 2 which acquires precipitate of the chondroitin sulfate which added ethanol in the concentration liquid of the chondroitin sulfate obtained by ultrafiltration processing, and was produced in it.
[Claim 5] The separation purification approach of chondroitin sulfate according to claim 1 to 4 that animal tissue is the cartilagines nasi of a salmon.
[Claim 6] The separation purification approach of chondroitin sulfate according to claim 1 to 5 of performing ultrafiltration processing twice [ at least ].
[Claim 7] claim 1 which adds water in chondroitin sulfate content liquid in advance of ultrafiltration processing thru/or 6 -- the separation purification approach given in either.

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DETAILED DESCRIPTION

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[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention carries out ultrafiltration processing of the chondroitin sulfate content water solution extracted from the connective tissue of the animal containing chondroitin sulfate, especially the cartilagines nasi of a salmon (salmon) head, performs concentration and purification of chondroitin sulfate continuously, and relates to the separation purification approach of chondroitin sulfate of obtaining chondroitin sulfate powder from concentration liquid. The chondroitin sulfate in which separation purification is carried out by this invention can be widely used for industrial products, such as a drugs raw material, a cosmetics raw material, and a food additive.
[0002]
[Background of the Invention] The salmon produced by the large quantity in recent years is conjointly eaten widely as a useful source of protein with the advance of refrigeration and a processing technique with hatching, fry culture, and a discharge technique. Although a salmon is fished by about 150,000t /, and a year and a large quantity in Hokkaido and the head and internal organs which are the processing wreckage are also discharged by the large quantity, except that the part is used as a raw material of a fish meal, it is hardly used, but the deployment is strongly desired from the marine-products-processing-industries company.
[0003] Chondroitin sulfate is contained in the cartilagines nasi of a salmon head, and although it is reported compared with conventional chondroitin sulfate that it is new chondroitin sulfate in which sulfuric-acid radical distribution has comparatively random structure (the collection of Chemical Society of Japan Hokkaido branch, such as Ayako etc. Sasaki, 1998 summer research presentation meeting lecture summaries, 23 pages), separation purification on industrial magnitude is not performed at all until now.
[0004] Chondroitin sulfate has the structure which the sulfuric-acid radical combined with the N-acetyl galactosamine on the basis of the disaccharide repetitive construct of glucuronic acid and N-acetyl galactosamine. Although the molecular weight changes with a raw material and methods of preparation, what was produced from a fillet of a shark, cartilago septi nasi of a calf, etc. which are acid tasteless odorless polysaccharide and are the main raw materials by which the current activity is carried out is used for a food additive, cosmetics, etc. taking advantage of the bioactive, water retention, and thickening nature about by tens of thousands to 300,000. Although the volume is about 200t per year, the cartilago septi nasi of that the fillet of a shark has few amounts of resources and a calf has a problem in the safeties of a raw material, such as the danger of an infectious disease (mad cow disease), and, as for all, it is in a difficult situation to stabilize and secure a cheap raw material.
[0005] As a separation purification method of the chondroitin sulfate in the water solution currently carried out industrially conventionally How to make it precipitate chondroitin sulfate by adding organic solvents, such as alcohol, in a chondroitin sulfate content water solution (K.Meyer, E.Davidson et, al, and Biochem.Biophys.Acta., 21, 506, 1956), The 4th class ammonium is added. As complex of water poor solubility How to settle and separate () [ Methods in ] Carbohydrate Chemistry, R.L.Whistler Acadimic Press, New York 5, 38, 1965, and the improving method (JP,1-210401,A) of those are indicated.
[0006] However, in order to obtain the chondroitin sulfate of a high grade to a large quantity by the above-mentioned approach, a lot of organic solvents (alcohol etc.) are required for generation and washing of precipitate, and in order for ******, such as protein, to precipitate simultaneously, it is necessary to use ion exchange resin etc. Moreover, by the approach using quarternary ammonium salt, the separation process for preventing mixing to the product of ammonium salt is needed.

[0007]
[Problem(s) to be Solved by the Invention] Therefore, the object of this invention is to establish the technique which can supply chondroitin sulfate to a large quantity cheaply by using as a raw material the animal tissue currently conventionally processed as trash, especially the salmon head (cartilagines-nasi part) produced in a large quantity as wreckage in a processing field. Furthermore, the object of this invention is to offer the separation purification method of the chondroitin sulfate which obtains the product of a high grade efficiently from an animal tissue, especially the chondroitin sulfate content water solution extracted from the cartilagines nasi of a salmon.
[0008]
[Means for Solving the Problem] this invention persons considered the concentration purification of impurity and the water solution containing chondroitin sulfate which carried out decomposition processing beforehand and carried out depolymerize of the components other than the chondroitin sulfate contained in the cartilagines nasi extracted from the head of a salmon (protein etc.). As a concentration purification method, by the pore of the ultrafiltration membrane side where the path was specified, the molecule of various magnitude could be exceeded and divided from the high molecular compound by the size of the pore of the film to a low molecular weight compound, and the approach using the ultrafiltration membrane put in practical use in the production process of dairy products, soy sauce, a seasoning, etc. in the food industry field was examined wholeheartedly. Consequently, using the ultrafiltration membrane of a specific cut off molecular weight, by carrying out multistage consecutive processing, it checks that the chondroitin sulfate of the various purity corresponding to an application is obtained efficiently, and came to complete this invention. In addition, although the approach of this invention is checked considering the cartilagines nasi of a salmon as a raw material, it is not restricted to the cartilagines nasi of a salmon, but can apply also to separation purification of the chondroitin sulfate under various tissues of other animals containing chondroitin sulfate widely.
[0009] Namely, the water solution which this invention carries out alkali treatment of the tissue of the animal containing 1 chondroitin sulfate, and contains chondroitin sulfate is obtained. The separation purification approach of the chondroitin sulfate characterized by carrying out ultrafiltration processing of this water solution, and performing concentration and purification of chondroitin sulfate, 2) The water solution which processes with proteolytic enzyme and contains chondroitin sulfate after alkali treatment is obtained. The separation purification approach of chondroitin sulfate given in said 1 which carries out ultrafiltration processing of the water solution, 3) The separation purification approach of chondroitin sulfate given in said 1 or 2 which obtains chondroitin sulfate powder by drying the concentration liquid of the chondroitin sulfate obtained by ultrafiltration processing, 4) The separation purification approach of chondroitin sulfate given in said 1 or 2 which acquires precipitate of the chondroitin sulfate which added ethanol in the concentration liquid of the chondroitin sulfate obtained by ultrafiltration processing, and was produced in it, 5) The separation purification approach of chondroitin sulfate given in either [ whose animal tissue is the cartilagines nasi of a salmon / said ] 1 thru/or 4, 6) — said 1 which adds water in chondroitin sulfate content liquid either [ which performs ultrafiltration processing twice / at least / said ] 1 thru/or 5 in advance of the separation purification approach of the chondroitin sulfate a publication, and 7 ultrafiltration processing thru/or 6 — either is provided with the separation purification approach of a publication.
[0010]
[The mode of implementation of invention] Hereafter, this invention is explained to a detail.
If it is the organization which contains chondroitin sulfate in abundance comparatively as an animal tissue raw material used by [chondroitin sulfate extraction feed] this invention, there will be no limit. Especially, conventionally, it is generated in a large quantity in a processing field etc., and the cartilagines nasi in the head of the fishes currently processed as a thing without utility value, especially a salmon is used preferably. Hereafter, the head (cartilagines nasi) of a salmon is mentioned as an example, and is explained.
[0011] From a [head end process] salmon head, the cartilagines nasi is extracted and the fragment (1-5mm angle extent) of this is carried out. Subsequently, in order to disassemble the protein contained in the cartilagines nasi, proteolytic enzyme processing is carried out alkali treatment and if needed. That is, first, after processing at the temperature of 37 degrees C thru/or 50 degrees C for 30 minutes to 3 hours in an alkali water solution (for example, caustic-alkali-of-sodium water solution (0.2-0.4N)), an acetic acid, a hydrochloric acid, etc. neutralize and filtration removes insoluble matter. Subsequently, pH of a filtrate is adjusted near neutrality, and after adding a proteolytic enzyme and processing around 40 degrees C for 1 hour to 2 hours, deactivation of the enzyme is carried out with heating. After cooling, centrifugal separation is carried out and the supernatant

liquid containing the depolymerize impurity produced in decomposition processing and chondroitin sulfate is obtained. Degree process carries out continuation multistage ultrafiltration processing of this supernatant liquid, and concentration and purification of chondroitin sulfate are performed simultaneously.

[0012] After having added 0.4-N sodium-hydroxide water solution so that the last concentration might be set to 0.2 Ns from a salmon head as one example at 13.2kg of cartilagines nasi which extracted the cartilagines nasi and carried out the fragment, and processing at 37 degrees C for 2 hours, the acetic acid neutralized and rough filtration removed insoluble matter. pH of a filtrate was prepared to 7.0, the protease (the product made from Amano Pharmaceuticals, trade name Amano A) was processed for 1 hour at 13.2g (0.1% of cartilagines-nasi weight) addition, and 37 degrees C, heating deactivation was carried out for 5 minutes at 85 degrees C, and 24l. (L) of supernatant liquid after centrifugal separation was obtained.

[0013] The outline of the ultrafilter used for continuation multistage ultrafiltration processing at [continuation multistage ultrafiltration processing] drawing 1 is shown. Among drawing, one is ultrafiltration membrane, contains an extract 2 on a tank 4, and carries out feeding supply with high pressure pumping 5 at ultrafiltration membrane 1. In this case, when the extract of the supernatant liquid obtained above was supplied to ultrafiltration membrane as it was, it became clear that chondroitin sulfate aiming at concentration fell out to a permeate liquid side, and the yield worsened. Then, what added water (tap water) 3 to supernatant liquid is supplied to ultrafiltration membrane. The addition of water should just carry out large purport tales-doses addition to supernatant liquid, although chondroitin sulfate is the amount no longer escaping from to a permeate liquid side.

[0014] Although what is necessary is just to select the ultrafiltration membrane to be used in consideration of the molecular weight of the chondroitin sulfate contained in raw material liquid, since the molecular weight is 20,000 (about 30,000-300,000) or more, in the case of the chondroitin sulfate contained in the salmon cartilagines nasi, the ultrafiltration membrane of a cut off molecular weight 20,000 should just be used. Although the average operating pressure of supply liquid sees the balance of the amount of transparency of the permeate liquid (7) which consists of the molecule and water below the discharge of the concentration liquid (6) containing the pressure resistance of the ultrafiltration membrane to be used, and the component more than the cut off molecular weight which passes ultrafiltration membrane, and a cut off molecular weight and is decided, it is 2-3kg/cm2 of large purports.

[0015] The 24l. (L) (chondroitin sulfate concentration about 17 g/L, about 30% of chondroitin acid purity.) of the above-mentioned extracts in addition, analysis of chondroitin sulfate -- some liquid -- extracting -- GARAMBOSU -- the amount of glucuronic acid was measured in law (Galambos JT 1967 The reaction of carbazole with carbohydrates 1.Effect of borate and sulfamate on the carbazole color of sugars.Anal.Biochem.19:119-132), and the quantum of the chondroitin sulfate concentration was carried out. chondroitin sulfate [ as opposed to a solid in purity ] -- comparatively -- the following -- being the same -- it received, the processing liquid which added water 24L was processed the condition for 10L/[ 50 degrees C and the mean pressure of 2kg/cm2 ], and about 37.0 permeate liquid L (4.4 times as many enrichment factor as this) was obtained (concentration of the 1st stage).

[0016] The chondroitin sulfate concentration in 31.8 g/L (purity is 63.5%) and permeate liquid of the chondroitin sulfate concentration in concentration liquid was 0.2 g/L (purity is 1.2%). Subsequently, addition mixing of the tap water of the amount of permeate liquid and tales doses was carried out, processing liquid was supplied from the tank 4 of drawing 1 , and ultrafiltration processing of the 2nd stage was performed similarly. The amount of permeate liquid is 36.4L, and chondroitin sulfate concentration was not accepted for the chondroitin sulfate concentration in concentration liquid into 32.5 g/L (purity is 88.4%) and permeate liquid.

[0017] Furthermore, the tap water of the amount of permeate liquid and tales doses was added, and ultrafiltration processing of the 3rd stage eye was performed similarly. The amount of permeate liquid is 35.6L, and obtained concentration liquid 12.0L. The chondroitin sulfate (CS) concentration in concentration liquid was not accepted for chondroitin sulfate into 31.6 g/L (purity is 98.6%) and permeate liquid.

[0018] The flow of the above result is shown in drawing 2 , and the analysis result of the extract before and behind each stage and concentration liquid is shown in a table 1.

[0019]

[A table 1]

A processing phase CS concentration CS purity g/l % Before processing An extract 16.8 30.4 The 1st stage Concentration liquid 31.8 63.5 Permeate liquid 0.2 1.2 The 2nd stage Concentration liquid 32.588.4 Permeate liquid 0 - The 3rd stage Concentration liquid 31.6 98.6 Permeate liquid 0 - [0020] Although runoff of the

chondroitin sulfate to permeate liquid was very small on each stage and the purity of chondroitin sulfate was 30.4% in the extract before processing so that clearly from the analysis result of the chondroitin sulfate concentration and purity of each processing liquid before and behind the membrane process of a table 1 Go up for every stage and it is refined to 98% or more on a stage 3. The chondroitin sulfate of a high grade can be obtained by drying the concentration liquid obtained by this as it is, or acquiring precipitate of an organic solvent and the chondroitin sulfate which added ethanol preferably and was produced.

[0021]

[Effect of the Invention] This invention removes with water low-molecular ******, such as a peptide which lives together by repeating ultrafiltration processing from the water solution containing the chondroitin sulfate obtained by carrying out depolymerize of the protein which extracts chondroitin sulfate with alkali from the cartilagines nasi of a salmon head, and lives together in an extract with proteolytic enzyme if needed, and provides concentration and coincidence of a water solution with a large quantity and the approach of performing efficiently for purification of chondroitin sulfate. Chondroitin sulfate can be obtained by condensing and refining a water solution by drying concentration liquid as it is, or settling chondroitin sulfate using organic solvents, such as little [ far ] ethanol, compared with the former. Furthermore, without ion-exchange resin etc., ** can also obtain the chondroitin sulfate of a high grade and reduction of cost is achieved.

[0022] When a salmon is fished by about 150,000t /, and a year and a large quantity in Hokkaido, about 15,000t (about 10% of cartilagines nasi is included) of heads which are the processing wreckage is discharged and chondroitin sulfate is manufactured to a large quantity, it is cheap, and it is the raw material stabilized dramatically. Furthermore, it is checked by the cartilagines nasi of a salmon head that acid polysaccharide other than the chondroitin sulfate looked at by the thing of other animal tissue origins (hyaluronic acid, the Delmer Than acid, heparan sulfate, etc.) hardly lives together.

[0023] It is possible to manufacture the chondroitin sulfate of a high grade (for remedies) from low purity (for example, for food additives) to arbitration by choosing the stage of a membrane process by this invention. Moreover, the powder of chondroitin sulfate can be obtained by drying concentration liquid as it is, without performing actuation which adds organic solvents, such as ethanol, in purification concentration liquid.

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## TECHNICAL FIELD

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EFFECT OF THE INVENTION

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[Effect of the Invention] This invention removes with water low-molecular ******, such as a peptide which lives together by repeating ultrafiltration processing from the water solution containing the chondroitin sulfate obtained by carrying out depolymerize of the protein which extracts chondroitin sulfate with alkali from the cartilagines nasi of a salmon head, and lives together in an extract with proteolytic enzyme if needed, and provides concentration and coincidence of a water solution with a large quantity and the approach of performing efficiently for purification of chondroitin sulfate. Chondroitin sulfate can be obtained by condensing and refining a water solution by drying concentration liquid as it is, or settling chondroitin sulfate using organic solvents, such as little [ far ] ethanol, compared with the former. Furthermore, without ion-exchange resin etc., ** can also obtain the chondroitin sulfate of a high grade and reduction of cost is achieved.
[0022] When a salmon is fished by about 150,000t /, and a year and a large quantity in Hokkaido, about 15,000t (about 10% of cartilagines nasi is included) of heads which are the processing wreckage is discharged and chondroitin sulfate is manufactured to a large quantity, it is cheap, and it is the raw material stabilized dramatically. Furthermore, it is checked by the cartilagines nasi of a salmon head that acid polysaccharide other than the chondroitin sulfate looked at by the thing of other animal tissue origins (hyaluronic acid, the Delmer Than acid, heparan sulfate, etc.) hardly lives together.
[0023] It is possible to manufacture the chondroitin sulfate of a high grade (for remedies) from low purity (for example, for food additives) to arbitration by choosing the stage of a membrane process by this invention. Moreover, the powder of chondroitin sulfate can be obtained by drying concentration liquid as it is, without performing actuation which adds organic solvents, such as ethanol, in purification concentration liquid.

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TECHNICAL PROBLEM

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[Problem(s) to be Solved by the Invention] Therefore, the object of this invention is to establish the technique which can supply chondroitin sulfate to a large quantity cheaply by using as a raw material the animal tissue currently conventionally processed as trash, especially the salmon head (cartilagines-nasi part) produced in a large quantity as wreckage in a processing field. Furthermore, the object of this invention is to offer the separation purification method of the chondroitin sulfate which obtains the product of a high grade efficiently from an animal tissue, especially the chondroitin sulfate content water solution extracted from the cartilagines nasi of a salmon.

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MEANS

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[Means for Solving the Problem] this invention persons considered the concentration purification of impurity and the water solution containing chondroitin sulfate which carried out decomposition processing beforehand and carried out depolymerize of the components other than the chondroitin sulfate contained in the cartilagines nasi extracted from the head of a salmon (protein etc.). As a concentration purification method, by the pore of the ultrafiltration membrane side where the path was specified, the molecule of various magnitude could be exceeded and divided from the high molecular compound by the size of the pore of the film to a low molecular weight compound, and the approach using the ultrafiltration membrane put in practical use in the production process of dairy products, soy sauce, a seasoning, etc. in the food industry field was examined wholeheartedly. Consequently, using the ultrafiltration membrane of a specific cut off molecular weight, by carrying out multistage consecutive processing, it checks that the chondroitin sulfate of the various purity corresponding to an application is obtained efficiently, and came to complete this invention. In addition, although the approach of this invention is checked considering the cartilagines nasi of a salmon as a raw material, it is not restricted to the cartilagines nasi of a salmon, but can apply also to separation purification of the chondroitin sulfate under various tissues of other animals containing chondroitin sulfate widely.
[0009] Namely, the water solution which this invention carries out alkali treatment of the tissue of the animal containing 1 chondroitin sulfate, and contains chondroitin sulfate is obtained. The separation purification approach of the chondroitin sulfate characterized by carrying out ultrafiltration processing of this water solution, and performing concentration and purification of chondroitin sulfate, 2) The water solution which processes with proteolytic enzyme and contains chondroitin sulfate after alkali treatment is obtained. The separation purification approach of chondroitin sulfate given in said 1 which carries out ultrafiltration processing of the water solution, 3) The separation purification approach of chondroitin sulfate given in said 1 or 2 which obtains chondroitin sulfate powder by drying the concentration liquid of the chondroitin sulfate obtained by ultrafiltration processing, 4) The separation purification approach of chondroitin sulfate given in said 1 or 2 which acquires precipitate of the chondroitin sulfate which added ethanol in the concentration liquid of the chondroitin sulfate obtained by ultrafiltration processing, and was produced in it, 5) The separation purification approach of chondroitin sulfate given in either [ whose animal tissue is the cartilagines nasi of a salmon / said ] 1 thru/or 4, 6) -- said 1 which adds water in chondroitin sulfate content liquid either [ which performs ultrafiltration processing twice / at least / said ] 1 thru/or 5 in advance of the separation purification approach of the chondroitin sulfate a publication, and 7 ultrafiltration processing thru/or 6 -- either is provided with the separation purification approach of a publication.
[0010]
[The mode of implementation of invention] Hereafter, this invention is explained to a detail.
If it is the organization which contains chondroitin sulfate in abundance comparatively as an animal tissue raw material used by [chondroitin sulfate extraction feed] this invention, there will be no limit. Especially, conventionally, it is generated in a large quantity in a processing field etc., and the cartilagines nasi in the head of the fishes currently processed as a thing without utility value, especially a salmon is used preferably. Hereafter, the head (cartilagines nasi) of a salmon is mentioned as an example, and is explained.
[0011] From a [head end process] salmon head, the cartilagines nasi is extracted and the fragment (1-5mm angle extent) of this is carried out. Subsequently, in order to disassemble the protein contained in the cartilagines nasi, proteolytic enzyme processing is carried out alkali treatment and if needed. That is, first, after processing at the temperature of 37 degrees C thru/or 50 degrees C for 30 minutes to 3 hours in an alkali water solution (for example, caustic-alkali-of-sodium water solution (0.2-0.4N)), an acetic acid, a hydrochloric acid,

etc. neutralize and filtration removes insoluble matter. Subsequently, pH of a filtrate is adjusted near neutrality, and after adding a proteolytic enzyme and processing around 40 degrees C for 1 hour to 2 hours, deactivation of the enzyme is carried out with heating. After cooling, centrifugal separation is carried out and the supernatant liquid containing the depolymerize impurity produced in decomposition processing and chondroitin sulfate is obtained. Degree process carries out continuation multistage ultrafiltration processing of this supernatant liquid, and concentration and purification of chondroitin sulfate are performed simultaneously.

[0012] After having added 0.4-N sodium-hydroxide water solution so that the last concentration might be set to 0.2 Ns from a salmon head as one example at 13.2kg of cartilagines nasi which extracted the cartilagines nasi and carried out the fragment, and processing at 37 degrees C for 2 hours, the acetic acid neutralized and rough filtration removed insoluble matter. pH of a filtrate was prepared to 7.0, the protease (the product made from Amano Pharmaceuticals, trade name Amano A) was processed for 1 hour at 13.2g (0.1% of cartilagines-nasi weight) addition, and 37 degrees C, heating deactivation was carried out for 5 minutes at 85 degrees C, and 24l. (L) of supernatant liquid after centrifugal separation was obtained.

[0013] The outline of the ultrafilter used for continuation multistage ultrafiltration processing at [continuation multistage ultrafiltration processing] drawing 1 is shown. Among drawing, one is ultrafiltration membrane, contains an extract 2 on a tank 4, and carries out feeding supply with high pressure pumping 5 at ultrafiltration membrane 1. In this case, when the extract of the supernatant liquid obtained above was supplied to ultrafiltration membrane as it was, it became clear that chondroitin sulfate aiming at concentration fell out to a permeate liquid side, and the yield worsened. Then, what added water (tap water) 3 to supernatant liquid is supplied to ultrafiltration membrane. The addition of water should just carry out large purport tales-doses addition to supernatant liquid, although chondroitin sulfate is the amount no longer escaping from to a permeate liquid side.

[0014] Although what is necessary is just to select the ultrafiltration membrane to be used in consideration of the molecular weight of the chondroitin sulfate contained in raw material liquid, since the molecular weight is 20,000 (about 30,000-300,000) or more, in the case of the chondroitin sulfate contained in the salmon cartilagines nasi, the ultrafiltration membrane of a cut off molecular weight 20,000 should just be used. Although the average operating pressure of supply liquid sees the balance of the amount of transparency of the permeate liquid (7) which consists of the molecule and water below the discharge of the concentration liquid (6) containing the pressure resistance of the ultrafiltration membrane to be used, and the component more than the cut off molecular weight which passes ultrafiltration membrane, and a cut off molecular weight and is decided, it is 2-3kg/cm2 of large purports.

[0015] The 24l. (L) (chondroitin sulfate concentration about 17 g/L, about 30% of chondroitin acid purity.) of the above-mentioned extracts in addition, analysis of chondroitin sulfate -- some liquid -- extracting -- GARAMBOSU -- the amount of glucuronic acid was measured in law (Galambos JT 1967 The reaction of carbazole with carbohydrates 1.Effect of borate and sulfamate on the carbazole color of sugars.Anal.Biochem.19:119-132), and the quantum of the chondroitin sulfate concentration was carried out. chondroitin sulfate [ as opposed to a solid in purity ] -- comparatively -- the following -- being the same -- it received, the processing liquid which added water 24L was processed the condition for 10L/[ 50 degrees C and the mean pressure of 2kg/cm2 ], and about 37.0 permeate liquid L (4.4 times as many enrichment factor as this) was obtained (concentration of the 1st stage).

[0016] The chondroitin sulfate concentration in 31.8g/L (purity is 63.5%), and permeate liquid of the chondroitin sulfate concentration in concentration liquid was 0.2 g/L (purity is 1.2%). Subsequently, addition mixing of the tap water of the amount of permeate liquid and tales doses was carried out, processing liquid was supplied from the tank 4 of drawing 1 , and ultrafiltration processing of the 2nd stage was performed similarly. The amount of permeate liquid is 36.4L, and chondroitin sulfate concentration was not accepted for the chondroitin sulfate concentration in concentration liquid into 32.5 g/L (purity is 88.4%) and permeate liquid.

[0017] Furthermore, the tap water of the amount of permeate liquid and tales doses was added, and ultrafiltration processing of the 3rd stage eye was performed similarly. The amount of permeate liquid is 35.6L, and obtained concentration liquid 12.0L. The chondroitin sulfate (CS) concentration in concentration liquid was not accepted for chondroitin sulfate into 31.6 g/L (purity is 98.6%) and permeate liquid.

[0018] The flow of the above result is shown in drawing 2 , and the analysis result of the extract before and behind each stage and concentration liquid is shown in a table 1.

[0019]

[A table 1]

A processing phase CS concentration CS purity g/l % Before processing An extract 16.8 30.4 The 1st stage Concentration liquid 31.8 63.5 Permeate liquid 0.2 1.2 The 2nd stage Concentration liquid 32.588.4 Permeate liquid 0 - The 3rd stage Concentration liquid 31.6 98.6 Permeate liquid 0 - [0020] Although runoff of the chondroitin sulfate to permeate liquid was very small on each stage and the purity of chondroitin sulfate was 30.4% in the extract before processing so that clearly from the analysis result of the chondroitin sulfate concentration and purity of each processing liquid before and behind the membrane process of a table 1 Go up for every stage and it is refined to 98% or more on a stage 3. The chondroitin sulfate of a high grade can be obtained by drying the concentration liquid obtained by this as it is, or acquiring precipitate of an organic solvent and the chondroitin sulfate which added ethanol preferably and was produced.

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[Translation done.]

* NOTICES *



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## DESCRIPTION OF DRAWINGS

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[Brief Description of the Drawings]
[Drawing 1] The outline of an ultrafilter is shown.
[Drawing 2] It is flow drawing of the example of this invention.
[Description of Notations]
1 Ultrafiltration Membrane
2 Extract
3 Water
4 Tank
5 High Pressure Pumping
6 Concentration Liquid
7 Permeate Liquid
8 Pressure Gage
9 Heat Exchanger
10 Flowmeter
11 Bulb

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[Translation done.]

* NOTICES *



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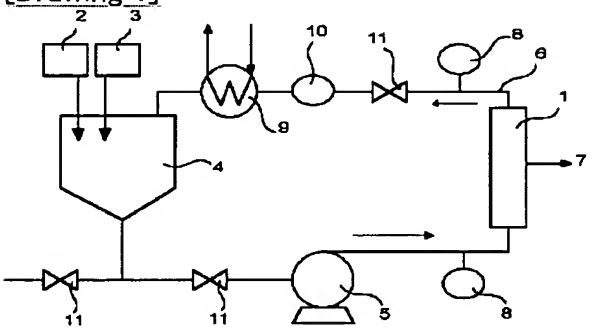
DRAWINGS

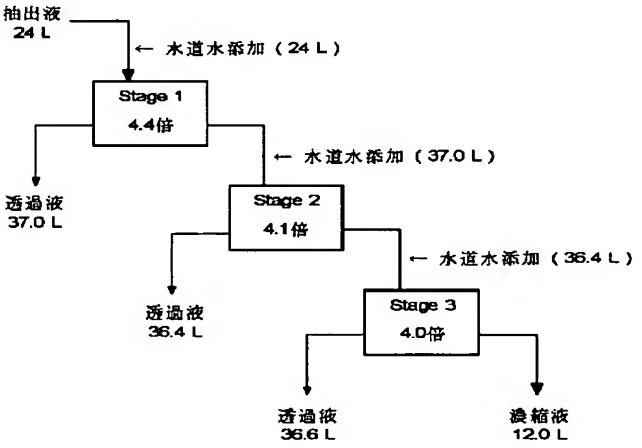
[Drawing 1]




[Drawing 2]

限外ろ過処理フロー

処理条件：分画分子量 2万、2 kg / cm$^2$ - 10 L / min.

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Ｆターム(参考)　4C090 AA04 BA66 BC28 CA16

(54)【発明の名称】　コンドロイチン硫酸の分離精製方法

(57)【要約】
【課題】　魚類の鼻軟骨、特にサケ頭部鼻軟骨を原料としてコンドロイチン硫酸を大量に安価に供給し得る技術を確立する。
【解決手段】　コンドロイチン硫酸を含む魚類の鼻軟骨をアルカリ処理し、必要に応じてタンパク分解酵素で処理してコンドロイチン硫酸を含有する水溶液を得、この水溶液を水で希釈した後、限外ろ過処理してコンドロイチン硫酸を濃縮する操作を反復して行ない、得られた濃縮液をそのまま乾燥するか、または濃縮液にエタノールを添加して生じたコンドロイチン硫酸の沈殿を取得してコンドロイチン硫酸粉末を得ることを特徴とするコンドロイチン硫酸の分離精製方法。

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【特許請求の範囲】

【請求項１】　コンドロイチン硫酸を含む動物の組織をアルカリ処理してコンドロイチン硫酸を含有する水溶液を得、この水溶液を限外ろ過処理してコンドロイチン硫酸の濃縮と精製を行なうことを特徴とするコンドロイチン硫酸の分離精製方法。

【請求項２】　アルカリ処理後にタンパク分解酵素で処理してコンドロイチン硫酸を含有する水溶液を得、その水溶液を限外ろ過処理する請求項１に記載のコンドロイチン硫酸の分離精製方法。

【請求項３】　限外ろ過処理により得られたコンドロイチン硫酸の濃縮液を乾燥することによりコンドロイチン硫酸粉末を得る請求項１または２に記載のコンドロイチン硫酸の分離精製方法。

【請求項４】　限外ろ過処理により得られたコンドロイチン硫酸の濃縮液にエタノールを添加して生じたコンドロイチン硫酸の沈殿を取得する請求項１または２に記載のコンドロイチン硫酸の分離精製方法。

【請求項５】　動物の組織がサケの鼻軟骨である請求項１乃至４のいずれかに記載のコンドロイチン硫酸の分離精製方法。

【請求項６】　限外ろ過処理を少なくとも２回行なう請求項１乃至５のいずれかに記載のコンドロイチン硫酸の分離精製方法。

【請求項７】　限外ろ過処理に先立ち、コンドロイチン硫酸含有液に水を添加する請求項１乃至６いずれかに記載の分離精製方法。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は、コンドロイチン硫酸を含む動物の結合組織、特にサケ（鮭）頭部の鼻軟骨より抽出したコンドロイチン硫酸含有水溶液を限外ろ過処理して連続的にコンドロイチン硫酸の濃縮と精製を行ない、濃縮液からコンドロイチン硫酸粉末を得るコンドロイチン硫酸の分離精製方法に関する。本発明により分離精製されるコンドロイチン硫酸は、医薬品原料、化粧品原料、食品添加物等の工業製品に広く利用することができる。

【０００２】

【発明の背景】孵化、稚魚養殖、放流技術により、近年大量に生産されるサケは、冷凍、加工技術の進歩と相俟って、有用な蛋白源として広く食されている。サケは北海道において約１５万トン／年と大量に漁獲され、その加工残骸である頭部や内臓も大量に排出されているが、その一部がフィッシュミールの原料として利用されている以外殆ど利用されておらず、その有効利用が水産加工業者から強く望まれている。

【０００３】サケ頭部の鼻軟骨にはコンドロイチン硫酸が含まれており、従来のコンドロイチン硫酸と比べ、硫酸基分布が比較的ランダムな構造を持つ新規なコンドロイチン硫酸であることが、報告されているが（佐々木綾子等，日本化学会北海道支部1998年夏期研究発表会講演要旨集，２３頁）、これまで、工業的規模での分離精製は全く行なわれていない。

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【０００４】コンドロイチン硫酸は、グルクロン酸とＮ－アセチルガラクトサミンの二糖繰返し構造を基本とし、そのＮ－アセチルガラクトサミンに硫酸基が結合した構造を有している。その分子量は原料及び調製法により異なるが、数万～３０万程度で、無味無臭の酸性多糖類であり、現在使用されている主な原料であるサメのヒレや子牛の鼻中隔軟骨などから生産されたものが、その生理活性、保水性、増粘性を生かして食品添加物や化粧品等に利用されている。その生産量は年間約２００トン程度であるが、サメのヒレは資源量が少ないこと、子牛の鼻中隔軟骨は感染症（狂牛病）の危険性など原料の安全性に問題があり、いずれも安価な原料を安定化して確保することが困難な状況にある。

【０００５】従来、工業的に実施されている水溶液中のコンドロイチン硫酸の分離精製法としては、アルコール等の有機溶媒をコンドロイチン硫酸含有水溶液に添加することによってコンドロイチン硫酸を沈析させる方法（K. Meyer, E. Davidson et,al, Biochem. Biophys. Acta., 21, 506, 1956）、第４級アンモニウムを添加して水難溶性の複合体として沈殿させ分離する方法（Methods in Carbohydrate Chemistry, R. L. Whistler Academic Press, New York 5, 38, 1965）及びその改良法（特開平1-210401号）が開示されている。

【０００６】しかしながら、上記方法で高純度のコンドロイチン硫酸を大量に得るためには、沈殿の生成及び洗浄に多量の有機溶媒（アルコール等）が必要であり、タンパク質等の爽雑物が同時に沈殿するためイオン交換樹脂などを使用する必要がある。また、第４級アンモニウム塩を用いる方法では、アンモニウム塩の製品への混入を防ぐための分離工程が必要となる。

【０００７】

【発明が解決しようとする課題】したがって、本発明の目的は、従来廃棄物として処理されている動物組織、特に加工場で残骸として大量に生じるサケ頭部（鼻軟骨部分）を原料としてコンドロイチン硫酸を大量に安価に供給し得る技術を確立することにある。さらに、本発明の目的は、動物組織、特にサケの鼻軟骨から抽出されるコンドロイチン硫酸含有水溶液から、高純度の製品を効率よく得るコンドロイチン硫酸の分離精製法を提供することにある。

【０００８】

【課題を解決するための手段】本発明者らは、サケの頭部から採取した鼻軟骨中に含まれるコンドロイチン硫酸以外の成分（蛋白質など）を予め分解処理して低分子化した、夾雑物とコンドロイチン硫酸を含有する水溶液の濃縮精製について検討した。濃縮精製法としては、径の

規定された限外ろ過膜面の細孔により高分子化合物から低分子化合物までの膜の細孔の大小により、種々の大きさの分子をこし分けることができ、食品産業分野で乳製品、醤油、調味料等の製造工程において実用化されている限外ろ過膜を用いる方法について鋭意検討した。その結果、特定の分画分子量の限外ろ過膜を用い、多段連続処理することにより、用途に対応した種々の純度のコンドロイチン硫酸が効率よく得られることを確認し、本発明を完成するに至った。なお、本発明の方法はサケの鼻軟骨を原料として確認されたものであるが、サケの鼻軟骨に限られず、コンドロイチン硫酸を含む他の動物の各種組織中のコンドロイチン硫酸の分離精製にも広く適用できるものである。

【０００９】すなわち、本発明は、

１）コンドロイチン硫酸を含む動物の組織をアルカリ処理してコンドロイチン硫酸を含有する水溶液を得、この水溶液を限外ろ過処理してコンドロイチン硫酸の濃縮と精製を行なうことを特徴とするコンドロイチン硫酸の分離精製方法、

２）アルカリ処理後にタンパク分解酵素で処理してコンドロイチン硫酸を含有する水溶液を得、その水溶液を限外ろ過処理する前記１に記載のコンドロイチン硫酸の分離精製方法、

３）限外ろ過処理により得られたコンドロイチン硫酸の濃縮液を乾燥することによりコンドロイチン硫酸粉末を得る前記１または２に記載のコンドロイチン硫酸の分離精製方法、

４）限外ろ過処理により得られたコンドロイチン硫酸の濃縮液にエタノールを添加して生じたコンドロイチン硫酸の沈殿を取得する前記１または２に記載のコンドロイチン硫酸の分離精製方法、

５）動物の組織がサケの鼻軟骨である前記１乃至４のいずれかに記載のコンドロイチン硫酸の分離精製方法、

６）限外ろ過処理を少なくとも２回行なう前記１乃至５のいずれかに記載のコンドロイチン硫酸の分離精製方法、および

７）限外ろ過処理に先立ち、コンドロイチン硫酸含有液に水を添加する前記１乃至６いずれかに記載の分離精製方法を提供するものである。

【００１０】

【発明の実施の態様】以下、本発明を詳細に説明する。

［コンドロイチン硫酸抽出原料］本発明で使用する動物組織原料としては、コンドロイチン硫酸を比較的豊富に含有する組織であれば制限はない。特に、従来、加工場などで大量に生じ、利用価値のないものとして処理されている魚類、特にサケの頭部中の鼻軟骨が好ましく用いられる。以下、サケの頭部（鼻軟骨）を例に挙げて説明する。

【００１１】［前処理工程］サケ頭部より鼻軟骨を採取し、これを細切（１～５ｍｍ角程度）する。次いで、鼻軟骨に含まれるタンパク質を分解するため、アルカリ処理および、必要に応じて蛋白分解酵素処理する。すなわち、まず、アルカリ水溶液（例えば、0.2～0.4Nの苛性ソーダ水溶液）中で３７℃乃至５０℃の温度で３０分～３時間処理した後、酢酸や塩酸等で中和し、不溶物をろ過により除去する。次いで、ろ液のｐＨを中性付近に調整して、蛋白分解酵素を添加して４０℃前後にて１時間～２時間処理した後、加熱により酵素を失活させる。冷却後、遠心分離して、分解処理で生じた低分子化夾雑物とコンドロイチン硫酸を含む上清を得る。この上清を次工程の連続多段限外ろ過処理してコンドロイチン硫酸の濃縮と精製を同時に行なう。



【００１２】一実施例としてサケ頭部より鼻軟骨を採取し、細切した鼻軟骨13.2ｋｇに最終濃度が0.2Nになるように0.4N水酸化ナトリウム水溶液を加え、３７℃で２時間処理した後、酢酸で中和し、粗ろ過により不溶物を除去した。ろ液のｐＨを7.0に調製し、プロテアーゼ（天野製薬(株）製、商品名アマノＡ）を13.2ｇ(鼻軟骨重量の0.1%）添加、３７℃で１時間処理し、８５℃で５分間加熱失活させて、遠心分離後の上清２４リットル（Ｌ）を得た。

【００１３】［連続多段限外ろ過処理］図１に、連続多段限外ろ過処理に使用した限外ろ過装置の概要を示す。図中、１は限外ろ過膜であり、抽出液２をタンク４に収納し高圧ポンプ５により限外ろ過膜１に圧送供給する。この場合、上記で得られた上清の抽出液をそのまま限外ろ過膜に供給すると、濃縮を目的とするコンドロイチン硫酸が透過液側に抜けて歩留まりが悪くなることが判明した。そこで、上清に対して水（水道水）３を添加したものを限外ろ過膜に供給する。水の添加量はコンドロイチン硫酸が透過液側に抜けなくなる量であるが、上清に対して大旨同量添加すればよい。

【００１４】使用する限外ろ過膜は、原料液に含まれるコンドロイチン硫酸の分子量を考慮して選定すればよいが、サケ鼻軟骨に含まれるコンドロイチン硫酸の場合はその分子量が２万以上（およそ３万～３０万）であることから、分画分子量２万の限外ろ過膜を使用すればよい。供給液の平均操作圧は、使用する限外ろ過膜の耐圧性と限外ろ過膜を通過する分画分子量以上の成分を含む濃縮液（６）の排出量および分画分子量以下の分子および水からなる透過液（７）の透過量のバランスをみて決められるが、大旨２～３ｋｇ／ｃｍ²である。

【００１５】上記抽出液２４リットル（Ｌ）（コンドロイチン硫酸濃度約１７ｇ／Ｌ，コンドロイチン酸純度約３０%。なお、コンドロイチン硫酸の分析は液の一部を採取し、ガランボス法（Galambos JT 1967 The reaction of carbazole with carbohydrates 1. Effect of borate and sulfamate on the carbazole color of sugars. Anal. Biochem. 19:119-132）にてグルクロン酸の量を測定してコンドロイチン硫酸濃度を定量した。純度は

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固形物に対するコンドロイチン硫酸の割合，以下同様）に対して、水２４Ｌを添加した処理液を、５０℃，平均圧力２ｋｇ／ｃｍ²，１０Ｌ／分の条件で処理し、透過液約37.0Ｌ（濃縮率4.4倍）を得た（第１ステージの濃縮）。

【００１６】濃縮液中のコンドロイチン硫酸濃度は31.8ｇ／Ｌ（純度は63.5%）、透過液中のコンドロイチン硫酸濃度は0.2ｇ／Ｌ（純度は1.2%）であった。次いで、透過液量と同量の水道水を添加混合し、図１のタンク４から処理液を供給して同様に第２ステージの限外ろ過処理を行なった。透過液量は36.4Ｌで、濃縮液中のコンドロイチン硫酸濃度は32.5ｇ／Ｌ（純度は88.4%）、透過＊

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＊液中にはコンドロイチン硫酸濃度は認められなかった。

【００１７】さらに、透過液量と同量の水道水を添加して、同様に第３ステージ目の限外ろ過処理を行なった。透過液量は35.6Ｌで、濃縮液12.0Ｌを得た。濃縮液中のコンドロイチン硫酸（ＣＳ）濃度は31.6ｇ／Ｌ（純度は98.6%）、透過液中にはコンドロイチン硫酸は認められなかった。

【００１８】以上の結果の流れを図２に示し、各ステージの前後の抽出液と濃縮液の分析結果を表１に示す。

【００１９】

【表１】

| 処理段階 | | ＣＳ濃度 ｇ／ｌ | ＣＳ純度 % |
|---|---|---|---|
| 処理前 | 抽出液 | 16.8 | 30.4 |
| 第１ステージ | 濃縮液 | 31.8 | 63.5 |
| | 透過液 | 0.2 | 1.2 |
| 第２ステージ | 濃縮液 | 32.5 | 88.4 |
| | 透過液 | 0 | － |
| 第３ステージ | 濃縮液 | 31.6 | 98.6 |
| | 透過液 | 0 | － |

【００２０】表１の膜処理前後の各処理液のコンドロイチン硫酸濃度と純度の分析結果から明らかなように、各ステージで透過液へのコンドロイチン硫酸の流出が極めて小さく、コンドロイチン硫酸の純度は、処理前の抽出液で30.4%であったが、各ステージごとに上昇し、ステージ３では９８%以上まで精製され、これによって得られた濃縮液をそのまま乾燥するか、あるいは有機溶剤、好ましくはエタノールを添加して生じたコンドロイチン硫酸の沈殿を取得するかして高純度のコンドロイチン硫酸を得ることができる。

【００２１】

【発明の効果】本発明は、サケ頭部の鼻軟骨よりコンドロイチン硫酸をアルカリで抽出し、必要に応じてタンパク分解酵素により抽出液中に共存するタンパク質を低分子化して得られたコンドロイチン硫酸を含有する水溶液から、限外ろ過処理を繰り返すことにより、共存するペプチド等の低分子爽雑物を水と共に除去し、水溶液の濃縮と同時にコンドロイチン硫酸の精製を、大量かつ効率的に行なう方法を提供したものである。水溶液を濃縮、精製することにより、濃縮液をそのまま乾燥するか、あるいは従来に比べてはるかに少ないエタノール等の有機溶媒を用いてコンドロイチン硫酸を沈殿させることによりコンドロイチン硫酸を得ることができる。さらにイオン交換樹脂等を使用せずとも高純度のコンドロイチン硫酸を得ることができ、コストの低減が図られる。

【００２２】サケは北海道において約１５万トン／年と大量に漁獲され、その加工残骸である頭部（鼻軟骨約１０%を含む）は約１万５千トン排出され、コンドロイチン硫酸を大量に製造する上で、安価で非常に安定した原料である。さらに、サケ頭部の鼻軟骨には他の動物組織由来のものに見られるコンドロイチン硫酸以外の酸性多糖体（ヒアルロン酸、デルマタン酸、ヘパラン硫酸など）が殆ど共存していないことが確認されている。

【００２３】本発明では、膜処理のステージを選択することで、低純度（例えば、食品添加物用）から高純度（医薬用）のコンドロイチン硫酸を任意に製造することが可能である。また、精製濃縮液にエタノールなどの有機溶剤を添加する操作を行なわずに濃縮液をそのまま乾燥することによって、コンドロイチン硫酸の粉末を得ることができる。

【図面の簡単な説明】

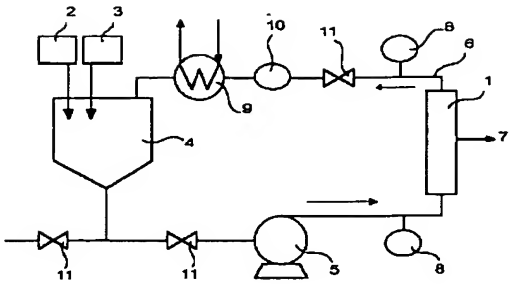
【図１】　限外ろ過装置の概要を示す。

【図２】　本発明の実施例のフロー図である。

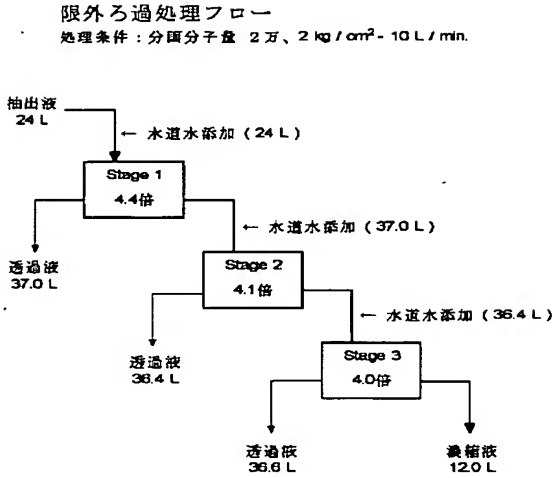
【符号の説明】

１　限外ろ過膜
２　抽出液
３　水
４　タンク
５　高圧ポンプ
６　濃縮液
７　透過液
８　圧力計
９　熱交換器
１０　流量計
１１　バルブ

【図１】




【図２】




【手続補正書】
【提出日】平成１２年３月１５日（２０００．３．１５）
【手続補正１】
【補正対象書類名】明細書
【補正対象項目名】特許請求の範囲
【補正方法】変更
【補正内容】
【特許請求の範囲】
【請求項１】　魚類の鼻軟骨をアルカリ処理してコンドロイチン硫酸を含有する水溶液を得、これに水を添加して希釈し限外ろ過により濃縮する工程を複数回繰り返すことによりコンドロイチン硫酸の精製を行なうことを特徴とするコンドロイチン硫酸の分離精製方法。
【請求項２】　アルカリ処理後にタンパク分解酵素で処理してコンドロイチン硫酸を含有する水溶液を得、その水溶液を限外ろ過処理する請求項１に記載のコンドロイチン硫酸の分離精製方法。
【請求項３】　限外ろ過処理により得られたコンドロイチン硫酸の濃縮液を乾燥することによりコンドロイチン硫酸粉末を得る請求項１または２に記載のコンドロイチン硫酸の分離精製方法。
【請求項４】　限外ろ過処理により得られたコンドロイチン硫酸の濃縮液にエタノールを添加して生じたコンドロイチン硫酸の沈殿を取得する請求項１または２に記載のコンドロイチン硫酸の分離精製方法。
【請求項５】　魚類の鼻軟骨がサケの鼻軟骨である請求項１乃至４のいずれかに記載のコンドロイチン硫酸の分離精製方法。
【請求項６】　分画分子量２万の限外ろ過膜を用いて限外ろ過処理を行なう請求項１乃至５のいずれかに記載のコンドロイチン硫酸の分離精製方法。
【請求項７】　コンドロイチン硫酸を含有する水溶液をタンク内にて水で希釈し、得られた希釈液を高圧ポンプを用いて限外ろ過装置に送出し、得られた濃縮液を前記タンクに循環することによりコンドロイチン硫酸の多段分離精製操作を連続的に行なう請求項１乃至６のいずれかに記載のコンドロイチン硫酸の分離精製方法。
【手続補正２】
【補正対象書類名】明細書
【補正対象項目名】０００１
【補正方法】変更
【補正内容】
【０００１】
【発明の属する技術分野】本発明は、コンドロイチン硫酸を含む動物の結合組織、具体的には魚類（例えば、サケ（鮭））頭部の鼻軟骨より抽出したコンドロイチン硫酸含有水溶液を限外ろ過処理して連続的にコンドロイチン硫酸の濃縮と精製を行ない、濃縮液からコンドロイチン硫酸粉末を得るコンドロイチン硫酸の分離精製方法に関する。本発明により分離精製されるコンドロイチン硫酸は、医薬品原料、化粧品原料、食品添加物等の工業製品に広く利用することができる。
【手続補正３】
【補正対象書類名】明細書
【補正対象項目名】０００７
【補正方法】変更

【補正内容】
【０００７】
【発明が解決しようとする課題】したがって、本発明の目的は、従来廃棄物として処理されている魚類頭部の鼻軟骨、特に加工場で残骸として大量に生じるサケ頭部（鼻軟骨部分）を原料としてコンドロイチン硫酸を大量に安価に供給し得る技術を確立することにある。さらに、本発明の目的は、魚類頭部の鼻軟骨、特にサケの鼻軟骨から抽出されるコンドロイチン硫酸含有水溶液から、高純度の製品を効率よく得るコンドロイチン硫酸の分離精製法を提供することにある。
【手続補正４】
【補正対象書類名】明細書
【補正対象項目名】０００８
【補正方法】変更
【補正内容】
【０００８】
【課題を解決するための手段】本発明者らは、魚類（例えば、サケ）の頭部から採取した鼻軟骨中に含まれるコンドロイチン硫酸以外の成分（蛋白質など）を予め分解処理して低分子化した、夾雑物とコンドロイチン硫酸を含有する水溶液の濃縮精製について検討した。濃縮精製法としては、径の規定された限外ろ過膜面の細孔により高分子化合物から低分子化合物までの膜の細孔の大小により、種々の大きさの分子をこし分けることができ、食品産業分野で乳製品、醤油、調味料等の製造工程において実用化されている限外ろ過膜を用いる方法について鋭意検討した。その結果、特定の分画分子量の限外ろ過膜を用い、多段連続処理することにより、用途に対応した種々の純度のコンドロイチン硫酸が効率よく得られることを確認し、本発明を完成するに至った。なお、本発明の方法はサケの鼻軟骨を原料として確認されたものであるが、サケの鼻軟骨に限られず、コンドロイチン硫酸を含む他の魚類の鼻軟骨中のコンドロイチン硫酸の分離精製にも広く適用できるものである。
【手続補正５】
【補正対象書類名】明細書
【補正対象項目名】０００９
【補正方法】変更
【補正内容】
【０００９】すなわち、本発明は、

１）魚類の鼻軟骨をアルカリ処理してコンドロイチン硫酸を含有する水溶液を得、これに水を添加して希釈し限外ろ過により濃縮する工程を複数回繰り返すことによりコンドロイチン硫酸の精製を行なうことを特徴とするコンドロイチン硫酸の分離精製方法。
２）アルカリ処理後にタンパク分解酵素で処理してコンドロイチン硫酸を含有する水溶液を得、その水溶液を限外ろ過処理する前記１に記載のコンドロイチン硫酸の分離精製方法。
３）限外ろ過処理により得られたコンドロイチン硫酸の濃縮液を乾燥することによりコンドロイチン硫酸粉末を得る前記１または２に記載のコンドロイチン硫酸の分離精製方法。
４）限外ろ過処理により得られたコンドロイチン硫酸の濃縮液にエタノールを添加して生じたコンドロイチン硫酸の沈殿を取得する前記１または２に記載のコンドロイチン硫酸の分離精製方法。
５）魚類の鼻軟骨がサケの鼻軟骨である前記１乃至４のいずれかに記載のコンドロイチン硫酸の分離精製方法。
６）分画分子量２万の限外ろ過膜を用いて限外ろ過処理を行なう前記１乃至５のいずれかに記載のコンドロイチン硫酸の分離精製方法。
７）コンドロイチン硫酸を含有する水溶液をタンク内にて水で希釈し、得られた希釈液を高圧ポンプを用いて限外ろ過装置に送出し、得られた濃縮液を前記タンクに循環することによりコンドロイチン硫酸の多段分離精製操作を連続的に行なう前記１～６のいずれかに記載のコンドロイチン硫酸の分離精製方法。
【手続補正６】
【補正対象書類名】明細書
【補正対象項目名】００１０
【補正方法】変更
【補正内容】
【００１０】
【発明の実施の態様】以下、本発明を詳細に説明する。
［コンドロイチン硫酸抽出原料］本発明で使用する動物組織原料としては、コンドロイチン硫酸を比較的豊富に含有する組織のうち、従来、加工場などで大量に生じ、利用価値のないものとして処理されている魚類、特にサケの頭部中の鼻軟骨が好ましく用いられる。以下、サケの頭部（鼻軟骨）を例に挙げて説明する。